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Synthesis of variously 9,9-dialkylated octahydropyrimido [3,4-*a*]-*s*-triazines with potential antifungal activity

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Abstract

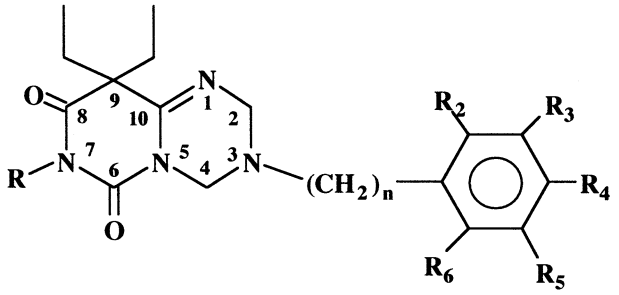
9,9-Dialkyloctahydropyrimido[3,4-*a*]-*s*-triazines were synthesized by iminodimethylation reaction between a 5,5-dialkyl-6-aminopyrimidine-2,4(3*H*,5*H*)-dione, a substituted aniline and two moles of formaldehyde. The synthesis of 5,5-dialkyl-6-aminopyrimidinedione consisted of the condensation of urea with ethyl 2,2-dialkylcyanoacetates. 18 Octahydropyrimido[3,4-*a*]-*s*-triazines were synthesized and compounds resulting from a supplementary aminomethylation were also obtained. Most of these compounds were tested for antifungal activity in vitro. Only 9,9-dibutyl-6,8-dioxo-3(2-chlorophenyl)2,3,4,5,6,7,8,9-octahydropyrimido[3,4-*a*]-*s*-triazine showed some activity against *Microsporum canis*. © 2002 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

Keywords: Iminodimethylation; Antifungal agents; Ethyl 2,2-dialkylcyanoacetates; 9,9-Dialkyloctahydropyrimido[3,4-*a*]-*s*-triazines

1. Introduction

The need for new pharmacophores which could be used to design new antifungal agents is still a concern for medicinal chemists.

Due to their structural relationship to both folic acids and cycloguanil ring, compounds containing the pharmacophore octahydropyrimido [3,4-*a*]-*s*-triazine have already been synthesized and proved to be active as antiparasitic [1,2] and antimicrobial agents [3].



Scheme 1.

In this last area, a pharmacomodulation has been performed only on the 9,9-diethylderivatives [4] (Scheme 1).

First of all, for $n = 0$, R = H, the phenyl group has been substituted by various substituents: Cl, NO_2, CH_3, OCH_3 in various positions. Preliminary antimicrobial results showing that chlorine was the more efficient substituent, the pharmacomodulation has been focussed on variation of the position and the number of Cl substituents on the phenyl group. The best antifungal results were obtained (MIC = 1 μml^{-1}) against *Trichophyton rubrum* and *Epidermophyton floccosum* for chlorine atom in position 3. In the case of bacteria a moderate activity has been observed for *Bacillus cereus*, when two chlorine atoms were introduced in positions 2 and 4 [4].

The alkylation of N7 by a methyl group, the replacement of the oxygen atom in position 6 by a sulfur atom, the introduction of methylene groups between N3 and the phenyl group, did not increase antifungal activity [4].

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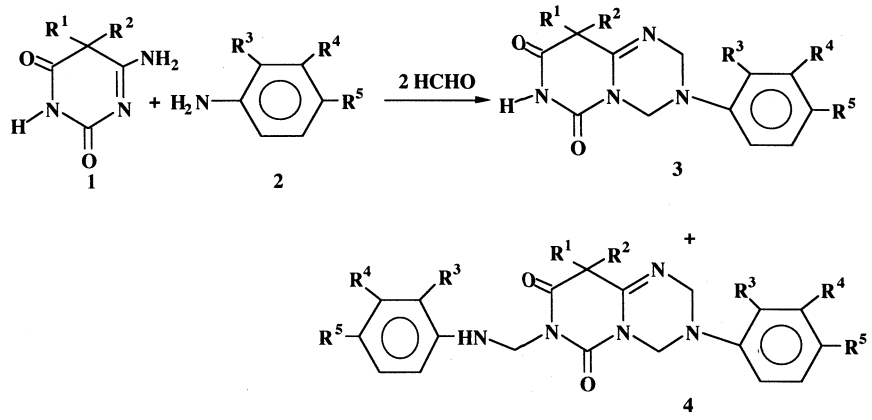


Scheme 2.

The present work deals with the modulation of the substituents of the carbon atom in position 9, in order to modify the lipophilicity of the compounds and to test its influence on the antifungal activity. Taking account of the results obtained with two ethyl groups in position 9, the substituents chosen for the phenyl group were one or two chlorine atoms, in various positions. Trifluoromethyl group, which is considered similar to chlorine from a pharmacological point of view, was also selected.

2. Results and discussion

2.1. Chemistry

The synthesis of octahydropyrimido[3,4-*a*]-*s*-triazines was performed via an iminodimethylation reaction of 5,5-dialkyl-6-aminopyrimidine-2,4(3*H*,5*H*)-dione, with formaldehyde and variously substituted anilines (Scheme 2). The 6-aminopyrimidinedione was under the enamino form and the condensation was an extension of the Mannich reaction [5].

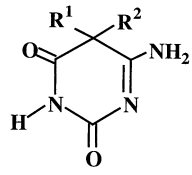
Four different 5,5-dialkyl-6-aminopyrimidine-2,4(3*H*,5*H*)-diones (**1a**–**d**) were used as starting materials (Table 1).

The first step of the synthesis consisted of the synthesis of ethyl 2,2-dialkylcyanoacetates. According to ancient works, the alkylation of ethyl cyanoacetate was performed by heating with alkyl halide and sodium ethanolate in stoichiometric conditions to yield monoalkylderivative, the second alkylation being obtained by the reaction of this monoalkyl derivative with a new molecule of alkylhalide in alkaline medium [6].

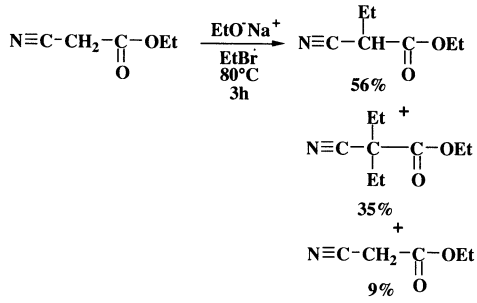
Unfortunately, under these conditions the result consisted always of a mixture of monoalkyl, of dialkyl derivatives and of starting material. Modifications of the relative proportions of cyanoacetate, alkyl halide or sodium ethanolate (1,1,1; 1,2,2; 1,1,2; 1,3,2) did not lead to a unique compound. For example, with one mole of sodium ethanolate and two moles of ethyl bromide, the major product was ethyl monoethylcyanoacetate (56%), but ethyl diethylcyanoacetate was obtained with 35% yield (Scheme 3).

Despite this lack of selectivity, this method could be used when a product dialkylated by the same alkyl group (Bu or Pr) was needed. In the presence of potassium hydroxide, the non-alkylated compound was a salt, which was very soluble in water, and could then be easily eliminated from the medium. For the monoalkylcompound, its solubility was weaker but

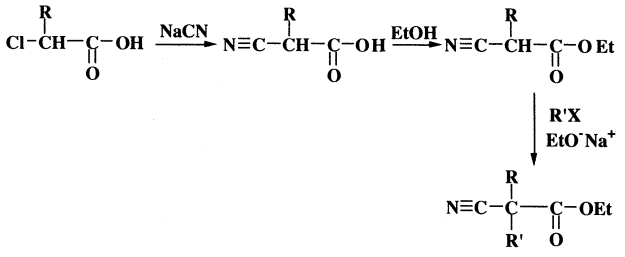
Table 1
5,5-Dialkyl-6-aminopyrimidine-2,4(3*H*,5*H*)-diones used as starting materials



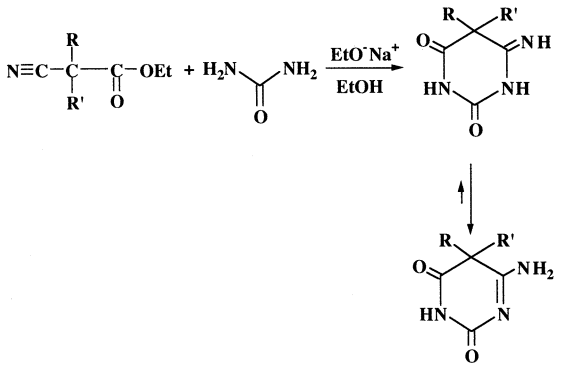
1	R^1	R^2
a	Bu	Bu
b	Pr	Pr
c	Bu	Et
d	Pr	Et



Scheme 3.

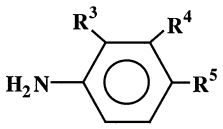


Scheme 4.



Scheme 5.

Table 2
Substituted anilines



2	R^3	R^4	R^5
a	H	H	H
b	Cl	H	H
c	H	Cl	H
d	H	H	Cl
e	Cl	H	Cl
f	CF_3	H	H
g	H	CF_3	H
h	H	H	CF_3

sufficient to allow a separation in alkaline medium. Dialkylcompound could then be isolated from the organic phase with 44% yield.

Unfortunately when the expected product was unsymmetrically dialkylated by two different alkyl groups, this method could not be used because the best yield after purification did not exceed 27%. Moreover, during the separation step of the monoalkylderivative by dissolution in alkaline medium, a hydrolysis of the ester group occurred, making it necessary to add a new esterification step to the sequence.

Another process adapted from the synthesis of ethyl cyanoacetate itself [7] had then to be used. The starting material was then constituted of α-chlorocarboxylic acid, which was treated with sodium cyanide, leading to monoalkylcyanoacetic acid, which was then submitted to an esterification reaction. The second alkylation was then obtained by treatment with an alkylhalide in the presence of sodium ethanolate (Scheme 4).

The following step consisted of the condensation of urea, with these ethyl dialkylcyanoacetates, in the presence of sodium ethanolate, according to the method described by Conrad [8] (Scheme 5).

The structure of this kind of compound which could undergo many kind of tautomerisms was studied by C. Giraud et al. [9] and the dioxoenamino form was proved to be predominant.

For the iminodimethylation reactions, these 6-aminopyrimidinediones reacted in ethanol solution with eight different anilines (1 mol) and formaldehyde (2 mol). These anilines were unsubstituted **2a** or variously substituted. Compounds **2b**–**d** were, respectively, substituted by a chlorine atom in positions 2, 3 or 4. Compound **2e** was substituted by two chlorine atoms in positions 2 and 4. Compounds **2f**–**h** were, respectively, substituted by a trifluoromethyl group in positions 2,3 or 4 (Table 2).

In each case, the product of the reaction was isolated after precipitation.

In most cases, octahydropyrimido[3,4-*a*]-*s*-triazines **3** were obtained. In sixteen cases, **3a**–**g**, **3i**–**p**, **3r**, were the only products of the reactions. In two cases the compounds resulting from an iminodimethylation **3h** and **3q** were accompanied, respectively, by **4a** and **4b**, resulting from a supplementary aminomethylation of the nitrogen atom in position 7. In four cases, the compounds **4c**, **4d**, **4e** and **4f** resulting from both an iminodimethylation and an aminomethylation were the only compounds obtained (Table 3).

All synthesized compounds (except **4a**, **4e** and **4f** for insolubility reasons) were tested against *Tricophyton mentagrophytes* (B70554), *Aspergillus fumigatus* (B42928), *Candida albicans* (B59630 and B63195), *Candida glabrata* (B63155), *Candida crusei* (B68404), *Candida parapsilosis* (B66126), *Candida kefyr* (81/018), *Candida tropicalis* (CDC44), *Cryptococcus neoformans* (B66663) and *Sporothrix schenckii* (B64284).

Against *Microsporum canis* (B68128), two more compounds **3a** and **4d** could not be essayed for insolubility reason under the experimental conditions.

In the case of *Tricophyton rubrum* (B68183), only **3d**, **3h**, **3o**, **3q** and **3r** were soluble enough to be tested under the experimental conditions.

All tested compounds failed to exhibit any antifungal activity except **3b** which presented a moderate activity against *Microsporum canis* ($0.4 < \mathrm{MIC} < 4 \times 10^{-6}$ g ml^{-1}).

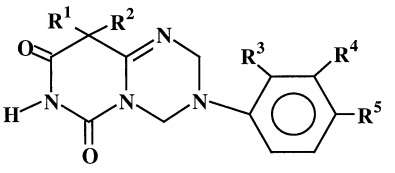
These results show that an increase of lipophilicity has a negative influence on antifungal activity, which could be related to a decrease of water solubility.

3. Experimental section

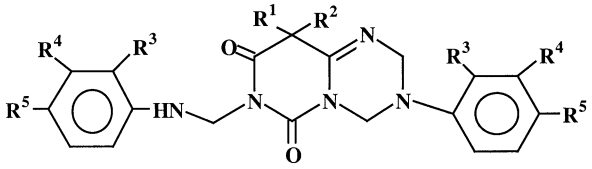
3.1. Chemistry

Melting points were determined on a Kofler apparatus and are uncorrected. Elemental analyses indicated by symbols were within $\pm 0.4\%$ of the theoretical values. ^{1}H NMR spectra were recorded on Bruker AC 200 spectrometer 200 MHz. A Hewlett Packard 5890 gas chromatograph equipped with a capillary column was used for the GC analyses. The fused silica column (25 m × 0.2 mmID × 0.11 μm film thickness) was coated with crosslinked 5% phenylmethyl silicon gum. The carrier gas was helium at an inlet pressure of 62 Kpa. Mass spectra were obtained on a GC–MS system. The mass spectrometer (Hewlett Packard 5970 MSD) was operating in electron impact mod (70 eV) and directly interfaced with the gas chromatograph apparatus.

Table 3
Octahydropyrimido[3,4-*a*]-*s*-triazines



3



4

Reactants	3	4	R^1	R^2	R^3	R^4	R^5
1a+2a	**a**		Bu	Bu	H	H	H
1a+2b	**b**		Bu	Bu	Cl	H	H
1a+2c	**c**		Bu	Bu	H	Cl	H
1a+2d	**d**		Bu	Bu	H	H	Cl
1a+2g	**e**		Bu	Bu	H	CF_3	H
1a+2h	**f**		Bu	Bu	H	H	CF_3
1b+2a	**g**		Pr	Pr	H	H	H
1b+2b	**h**	**a**	Pr	Pr	Cl	H	H
1b+2c	**i**		Pr	Pr	H	Cl	H
1b+2d	**j**		Pr	Pr	H	H	Cl
1b+2e	**k**		Pr	Pr	Cl	H	Cl
1b+2f	**l**		Pr	Pr	CF3	H	H
1c+2a	**m**		Bu	Et	H	H	H
1c+2c	**n**		Bu	Et	H	Cl	H
1c+2d	**o**		Bu	Et	H	H	Cl
1d+2a	**p**		Pr	Et	H	H	H
1d+2b	**q**	**b**	Pr	Et	Cl	H	H
1d+2c	**r**		Pr	Et	H	Cl	H
1b+2g		**c**	Pr	Pr	H	CF_3	H
1b+2h		**d**	Pr	Pr	H	H	CF_3
1c+2e		**e**	Bu	Et	Cl	H	Cl
1d+2d		**f**	Pr	Et	H	H	Cl

Table 4
Mono- and dialkylated ethyl cyanoacetates (yields and Ref.)

R^1	R^2	Yield (%)	Ref.
Et	H	18	[11]
Pr	H	27	[12]
Bu	H	22	[11]
Pr	Pr	16	[8]
Pr	Bu	22	[13]
Bu	Et	83	[8]
Bu	Et	78	[14]

3.1.1. Synthesis of ethyl 2,2-dialkylcyanoacetates

3.1.1.1. Classical synthesis. The method used was described by Traube [6]: sodium (23 g,1 mol) was dissolved in 400 ml dry ethanol [10]. After cooling, distilled ethyl cyanacetate (106 ml, 1 mol) was added, then alkylhalide (1 mol) was added dropwise. The mixture was stirred at room temperature for 3 h. After the solvent was evaporated and the residue was dissolved in the minimal volume of water, two phases were obtained. The aqueous phase was extracted with diethyloxide (3 × 57 ml). The organic phase was dried, the solvent was evaporated and the residue was purified by distillation under reduced pressure. The different compounds were quantified by GC–MS. For R = ethyl and with ethyl bromide (2 mol), the yields were: ethyl monoethylcyanoacetate 56%; ethyl diethylcyanoacetate 35%; non alkylated ethylcyanoacetate 9%.

In order to obtain pure monoalkyl and dialkylderivatives, the distillate was first washed with aliquot quantities of sodium hydroxide (20% in water) corresponding to the amount on non-alkylated compound present in the reaction medium. The aqueous phase was eliminated. Then the organic residue was washed again with aliquot quantities of sodium hydroxide corresponding to the amounts of monoalkylated compound present. Dialkylated compounds remained in the organic phase and were separated by distillation under reduced pressure. The aqueous phase was evaporated under vacuum, then the residue was dissolved with the minimal amounts of water. Hydrochloric acid was added until two phases appeared. The organic phase was constituted of monoalkylcyanoacetic acid. This acid was esterified with an excess of ethanol and ten drops of sulfuric acid. The ethyl monoalkylcyanoacetate was distilled under reduced pressure. Table 4 reports the yields of these compounds.

3.1.1.2. Alternative synthesis of unsymmetrically dialkylated ethyl 2,2-dialkyl cyanoacetates from α-chlorocarboxylic acids. Synthesis of ethyl 2-alkylcyanoacetates was realized according to the method described in organic syntheses [7] for ethylcyanoacetate itself, using

2-chloroalkylcarboxylic acids instead of chloroacetic acid.

The second alkylation was performed by the classical method described in [6].

Boiling points were in agreement with the literature. N.B. When the compounds were chiral they were isolated as racemates.

3.1.2. Synthesis of 5,5-dialkyl-6-aminopyrimidine-2,4(3H,5H)-diones

5,5-Dialkyl-6-aminopyrimidine-2,4(3*H*,5*H*)-diones were prepared according to the standard method: condensation of urea with the appropriate ethyl 2,2-dialkylcyanoacetate in alkaline medium [7].

3.1.2.1. 6-Amino-5,5-dibutylpyrimidine-2,4(3H,5H)dione ***(1a)***. Yield: 32%; m.p. > 260 °C; *Anal.* (C, H, N) $C_{12}H_{21}N_3O_2$. ^{1}H-NMR (DMSO-d_6) δ ppm: 0.8 (t, 6H, two CH_3 butyl); 0.95 (m, 4H, $CH_3CH_2CH_2CH_2$); 1.15 (m, 4H, $CH_3CH_2CH_2CH_2$–); 1.8 (t, 4H, $CH_3CH_2CH_2CH_2$–); 7.65 (s, 1H, ech D_2O, NH_2); 7.95 (s, 1H,ech D_2O, NH_2); 10.5 (s, 1H, ech D_2O, C=O–N*H*–C=O).

3.1.2.2. 6-Amino-5-,5-dipropylpyrimidine-2,4(3H,5H)-dione ***(1b)***. Yield: 44%; m.p. > 260 °C; *Anal.* (C, H, N) $C_{10}H_{17}N_3O_2$. ^{1}H NMR (DMSO-d_6) δ ppm: 0.8 (t, 6H, two CH_3 propyl); 1.05 (m, 4H, $CH_3CH_2CH_2$–); 1.8 (m, 4H, $CH_3CH_2CH_2$–); 7.9 (s, 1H, ech D_2O,NH_2); 8.35 (s, 1H,ech D_2O, NH_2); 10.6 (s, 1H, ech D_2O, C=O–N*H*–C=O).

3.1.2.3. 6-Amino-5-butyl-5-ethylpyrimidine-2,4(3H,5H)-dione ***(1c)***. Yield: 47%; m.p. > 260 °C; *Anal.* (C, H, N) $C_{10}H_{17}N_3O_2$. ^{1}H NMR (DMSO-d_6) δ ppm: 0.8 (t, 6H, two CH_3); 1.1 (m, 4H, $CH_3CH_2CH_2CH_2$–); 1.9 (m, 4H, CH_3CH_2– and $CH_3CH_2CH_2CH_2$–); 8.0 (s,1H, ech D_2O, NH_2); 8.46 (s, 1H, ech D_2O, NH_2); 10.1 (s, 1H, ech D_2O, C=O–N*H*–C=O).

3.1.2.4. 6-Amino-5-ethyl-5-propylpyrimidine-2,4(3H,5H)dione ***(1d)***. Yield: 41%; m.p. > 260 °C; *Anal.* (C, H, N) $C_9H_{15}N_3O_2$. ^{1}H NMR (DMSO-d_6) δ ppm: 0.65 (t, 3H, CH_3 propyl); 0.8 (t, 3H, CH_3 ethyl); 1.05 (m, 2H, $CH_3CH_2CH_2$–); 1.9 (m, 4H, CH_3CH_2– and $CH_3CH_2CH_2$); 7.95 (s, 1H, ech D_2O, NH_2); 8.45 (s, 1H,ech D_2O, NH_2); 10.6 (s, 1H, ech D_2O, C=O–N*H*–C=O).

3.1.3. Synthesis of 9,9-dialkyloctahydropyrimido[3,4-a]-s-triazines

5,5-Dialkyl-6-aminopyrimidine-2,4(3*H*,5*H*)-dione (0.015 mol) was added to formaldehyde (0.03 mol) and variously substituted anilines (0.015 mol) in 10 ml absolute ethanol. The mixture was stirred at reflux of ethanol for 30 min. After cooling, the octahydropyrimido[3,4-*a*]-*s*-triazine **3** or **4** was filtered and then dried.

When **3** and **4** both precipitated (**3h** and **4a**; **3q** and **4b**), they were dissolved in ethyl oxide and **4** was precipitated by acetone.

3.1.3.1. 9,9-Dibutyl-6,8-dioxo-3-phenyl-2,3,4,5,6,7,8,9-octahydropyrimido[3,4-a]-s-triazine ***(3a)***. Yield: 71%; m.p.: 149 °C, *Anal.* (C, H, N) $C_{20}H_{28}N_4O_2$. ^{1}H NMR ($CDCl_3$) δ ppm: 0.7 (t, 6H, 2 CH_3 butyl); 0.95 (m, 4H, $CH_3CH_2CH_2CH_2$–); 1.15 (m, 4H, $CH_3CH_2CH_2CH_2$–); 1.85 (m, 4H, $CH_3CH_2CH_2CH_2$–); 5.0 (s, 2H, N–CH_2–N); 5.35 (s, 2H, C=O–N–CH_2–N); 6.95 (m, 3H, Ar); 7.25 (m, 2H, Ar); 8.2 (s, 1H, ech D_2O, C=O–NH–C=O).

3.1.3.2. 9,9-Dibutyl-6,8-dioxo-3(2-chlorophenyl)-2,3,4,5,6,7,8,9-octahydropyrimido[3,4-a]-s-triazine ***(3b)***. Yield: 80%; m.p.: 164 °C, *Anal.* (C, H, N) $C_{20}H_{27}N_4O_2Cl$. ^{1}H NMR ($CDCl_3$) δ ppm: 0.8 (t, 6H, 2 CH_3 butyl); 1.2 (m, 8H, $CH_3CH_2CH_2CH_2$–), 1.9 (m, 4H, $CH_3CH_2CH_2CH_2$–); 4.95 (s, 2H, N–CH_2–N); 5.3 (s, 2H, C=O–N–CH_2–N); 7.05 (m, 3H, Ar); 7.35 (d, 1H, Ar); 8.05 (s, 1H, ech D_2O, C=O–NH–C=O).

3.1.3.3. 9,9-Dibutyl-6,8-dioxo-3(3-chlorophenyl)-2,3,4,5,6,7,8,9-octahydropyrimido[3,4-a]-s-triazine ***(3c)***. Yield: 22%; m.p.: 132 °C, *Anal.* (C, H, N) $C_{20}H_{27}N_4O_2Cl$. ^{1}H NMR ($CDCl_3$) δ ppm: 0.75 (t, 6H, 2 CH_3 butyl); 0.95 (m, 4H, $CH_3CH_2CH_2CH_2$–); 1.15 (m, 4H, $CH_3CH_2CH_2CH_2$–); 1.85 (m, 4H, $CH_3CH_2CH_2CH_2$–); 5.0 (s, 2H, N–CH_2–N); 5.35 (s, 2H, C=O–N–CH_2–N); 6.9 (m, 3H, Ar); 7.25 (m, 1H, Ar); 8.45 (s, 1H, ech D_2O, C=O–NH–C=O).

3.1.3.4. 9,9-Dibutyl-6,8-dioxo-3(4-chlorophenyl)-2,3,4,5,6,7,8,9-octahydropyrimido[3,4-a]-s-triazine ***(3d)***. Yield: 81%; m.p.: 142 °C, *Anal.* (C, H, N) $C_{20}H_{27}N_4O_2Cl$. ^{1}H NMR ($CDCl_3$) δ ppm: 0.75 (t, 6H, 2 CH_3 butyl); 1.0 (m, 4H, $CH_3CH_2CH_2CH_2$–); 1.15 (m, 4H, $CH_3CH_2CH_2CH_2$–); 1.9 (m, 4H, $CH_3CH_2CH_2CH_2$–); 5.05 (s, 2H, N–CH_2–N); 5.45 (s, 2H, C=O–N–CH_2–N); 6.95 (d, 2H, Ar); 7.25 (d, 1H, Ar); 8.45 (s, 1H, ech D_2O, C=O–NH–C=O).

3.1.3.5. 9,9-Dibutyl-6,8-dioxo-3(3-trifluoromethylphenyl)-2,3,4,5,6,7,8,9 octahydropyrimido[3,4-a]-s-triazine ***(3e)***. Yield: 15%; m.p.: 135 °C, *Anal.* (C, H, N) $C_{21}H_{27}N_4O_2F_3$. ^{1}H NMR ($CDCl_3$) δ ppm: 0.7 (t, 6H, 2 CH_3 butyl); 0.85 (m, 4H, $CH_3CH_2CH_2CH_2$–); 1.1 (m, 4H, $CH_3CH_2CH_2CH_2$–); 1.9 (m, 4H, $CH_3CH_2CH_2CH_2$–); 5.05 (s, 2H, N–CH_2–N); 5.4 (s, 2H, C=O–N–CH_2–N); 7.15 (m, 3H, Ar); 7.4 (t, 1H, Ar); 9.15 (s, 1H, ech D_2O, C=O–NH–C=O).

3.1.3.6. 9,9-Dibutyl-6,8-dioxo-3(4-trifluoromethylphenyl)-2,3,4,5,6,7,8,9-octahydropyrimido[3,4-a]-s-triazine ***(3f)***. Yield: 2%; m.p.: 129 °C, *Anal.* (C, H, N)

$C_{21}H_{27}N_4O_2F_3$. ^1H NMR ($CDCl_3$) δ ppm: 0.65 (t, 6H, two CH_3 butyl); 0.85 (m, 4H, $CH_3CH_2CH_2CH_2$–); 1.05 (m, 4H, $CH_3CH_2CH_2CH_2$–); 1.8 (m, 4H, CH_3CH_2-CH_2CH_2–); 5.05 (s, 2H, N–CH_2–N); 5.35 (s, 2H, C=O–N–CH_2–N); 7.05 (d, 2H, Ar); 7.5 (d, 2H, Ar); 8.4 (s, 1H, ech D_2O, C=O–NH–C=O).

3.1.3.7. 9,9-Dipropyl-6,8-dioxo-3-phenyl-2,3,4,5,6,7,8,9-octahydropyrimido[3,4-a]-s-triazine ***(3g)***. Yield: 67%; m.p.: 184 °C, *Anal.* (C, H, N) $C_{18}H_{24}N_4O_2$. ^1H NMR ($CDCl_3$) δ ppm: 0.75 (t, 6H, two CH_3 propyl); 1.1 (m, 4H, $CH_3CH_2CH_2$–); 1.85 (m, 4H, $CH_3CH_2CH_2$–); 5.0 (s, 2H, N–CH_2–N); 5.35 (s, 2H, C=O–N–CH_2–N); 7.0 (t,2H, Ar); 7.3 (m., 2H, Ar); 8.75 (s, 1H, ech D_2O, C=O–NH–C=O).

3.1.3.8. 9,9-Dipropyl-6,8-dioxo-3-(2-chlorophenyl)-2,3,4,5,6,7,8,9-octahydropyrimido[3,4-a]-s-triazine ***(3h)***. Yield: 13%; m.p.: 215 °C, *Anal.* (C, H, N) $C_{18}H_{23}N_4O_2Cl$. ^1H NMR ($CDCl_3$) δ ppm: 0.85 (t, 6H, two CH_3 propyl); 1.2 (m, 4H, $CH_3CH_2CH_2$–); 1.95 (m, 4H, $CH_3CH_2CH_2$–); 5.0 (s, 2H, N–CH_2–N); 5.3 (s, 2H, C=O–N–CH_2–N); 7.1 (m, 2H, Ar); 7.2 (m, 1H, Ar); 7.45 (d, 1H, Ar); 8.45 (s, 1H, ech D_2O, C=O–NH–C=O).

3.1.3.9. 9,9-Dipropyl-6,8-dioxo-3-(3-chlorophenyl)-2,3,4,5,6,7,8,9-octahydropyrimido[3,4-a]-s-triazine ***(3i)***. Yield: 45%; m.p.: 156 °C, *Anal.* (C, H, N) $C_{18}H_{23}N_4O_2Cl$. ^1H NMR ($CDCl_3$) δ ppm: 0.75 (t, 6H, two CH_3 propyl); 1.05 (m, 4H, $CH_3CH_2CH_2$–); 1.85 (m, 4H, $CH_3CH_2CH_2$–); 4.95 (s, 2H, N–CH_2–N); 5.3 (s, 2H, C=O–N–CH_2–N); 6.9 (m, 3H, Ar); 7.15 (m, 1H, Ar); 8.8 (s, 1H, ech D_2O, C=O–NH–C=O).

3.1.3.10. 9,9-Dipropyl-6,8-dioxo-3-(4-chlorophenyl)-2,3,4,5,6,7,8,9-octahydropyrimido[3,4-a]-s-triazine ***(3j)***. Yield: 66%; m.p.: 169 °C, *Anal.* (C, H, N) $C_{18}H_{23}N_4O_2Cl$. ^1H NMR ($CDCl_3$) δ ppm: 0.75 (t, 6H, two CH_3 propyl); 1.05 (m, 4H, $CH_3CH_2CH_2$–); 1.85 (m, 4H, $CH_3CH_2CH_2$–); 4.95 (s, 2H, N–CH_2–N); 5.35 (s, 2H, C=O–N–CH_2–N); 6.9 (d, 2H, Ar); 7.2 (d, 2H, Ar); 8.6 (s, 1H, ech D_2O, C=O–NH–C=O).

3.1.3.11. 9,9-Dipropyl-6,8-dioxo-3-(2,4-dichlorophenyl)-2,3,4,5,6,7,8,9-octahydropyrimido [3,4-a]-s-triazine ***(3k)***. Yield: 3%; m.p.: 175 °C, *Anal.* (C, H, N) $C_{18}H_{22}N_4O_2Cl_2$. ^1H NMR ($CDCl_3$) δ ppm: 0.8 (t, 6H, two CH_3 propyl); 1.1 (m, 4H, $CH_3CH_2CH_2$–); 1.9 (m, 4H, $CH_3CH_2CH_2$–); 4.9 (s, 2H, N–CH_2–N); 5.25 (s, 2H, C=O–N–CH_2–N); 6.85 (d, 1H, Ar); 7.1 (dd,1H, Ar); 7.4 (d,1H, Ar); 8.05 (s, 1H, ech D_2O, C=O–NH–C=O).

3.1.3.12. 9,9-Dipropyl-6,8-dioxo-3-(2-trifluoromethylphenyl)-2,3,4,5,6,7,8,9-octahydropyrimido[3,4-a]-s-triazine ***(3l)***. Yield: 28%; m.p.: 176 °C, *Anal.* (C, H, N) $C_{19}H_{23}N_4O_2F_3$. ^1H NMR ($CDCl_3$) δ ppm: 0.85 (t, 6H, two CH_3 propyl); 1.2 (m, 4H, $CH_3CH_2CH_2$–); 1.9 (m, 4H, $CH_3CH_2CH_2$–); 4.85 (s, 2H, N–CH_2–N); 5.1 (s, 2H, C=O–N–CH_2–N); 7.1 (d, 1H, Ar); 7.25 (t,1H, Ar); 7.45 (t, 1H, Ar); 7.65 (d,1H, Ar); 8.1 (s, 1H, ech D_2O, C=O–NH–C=O).

3.1.3.13. 9-Butyl-9-ethyl-6,8-dioxo-3-phenyl-2,3,4,5,6,7,8,9-octahydropyrimido[3,4-a]-s-triazine ***(3m)***. Yield: 19%; m.p.: 168 °C, *Anal.* (C, H, N) $C_{18}H_{24}N_4O_2$. ^1H NMR ($CDCl_3$) δ ppm: 0.7 (t, 6H, 2 CH_3); 1.0 (m, 4H, $CH_3CH_2CH_2CH_2$–); 2.0 (t, 4H, CH_3CH_2– and $CH_3CH_2CH_2CH_2$–); 5.2 (s, 2H, N–CH_2–N); 5.6 (s, 2H, C=O–N–CH_2–N); 7.4 (m, 5H, Ar); 8.4 (s, 1H, ech D_2O, C=O–NH–C=O).

3.1.3.14. 9-Butyl-9-ethyl-6,8-dioxo-3-(3-chlorophenyl)-2,3,4,5,6,7,8,9-octahydropyrimido [3,4-a]-s-triazine ***(3n)***. Yield: 51%; m.p.: 152 °C, *Anal.* (C, H, N) $C_{18}H_{23}N_4O_2Cl$. ^1H NMR ($CDCl_3$) δ ppm: 0.7 (t, 6H, 2 CH_3); 1.1 (m, 4H, $CH_3CH_2CH_2CH_2$–); 1.9 (t, 4H, CH_3CH_2– and $CH_3CH_2CH_2CH_2$–); 5.1 (s, 2H, N–CH_2–N); 5.4 (s, 2H, C=O–N–CH_2–N); 7.4 (m, 4H, Ar); 8.4 (s, 1H, ech D_2O, C=O–NH–C=O).

3.1.3.15. 9-Butyl-9-ethyl-6,8-dioxo-3-(4-chlorophenyl)-2,3,4,5,6,7,8,9-octahydropyrimido [3,4-a]-s-triazine ***(3o)***. Yield: 24%; m.p.: 138 °C, *Anal.* (C, H, N) $C_{18}H_{23}N_4O_2Cl$. ^1H NMR ($CDCl_3$) δ ppm: 0.7 (t, 6H, 2 CH_3); 0.95 (m, 2H,$CH_3CH_2CH_2CH_2$–); 1.1 (m, 2H,$CH_3CH_2CH_2CH_2$–); 1.9 (t, 4H, CH_3CH_2– and $CH_3CH_2CH_2CH_2$–); 5.0 (s, 2H, N–CH_2–N); 5.3 (dd, 2H, C=O–N–CH_2–N); 6.9 (d, 2H, Ar); 7.2 (d, 2H, Ar); 9.35 (s, 1H, ech D_2O, C=O–NH–C=O).

3.1.3.16. 9-Ethyl-9-propyl-6,8-dioxo-3-phenyl-2,3,4,5,6,7,8,9-octahydropyrimido [3,4-a]-s-triazine ***(3p)***. Yield: 19%; m.p.: 164 °C, *Anal.* (C, H, N) $C_{17}H_{22}N_4O_2$. ^1H NMR ($CDCl_3$) δ ppm: 0.8 (t, 6H, 2 CH_3); 1.0 (m, 2H,$CH_3CH_2CH_2$–); 1.9 (t, 4H, CH_3CH_2– and $CH_3CH_2CH_2$–); 5.2 (s, 2H, N–CH_2–N); 5.6 (s, 2H, C=O–N–CH_2–N); 7.3 (m, 5H, Ar); 9.4 (s, 1H, ech D_2O, C=O–NH–C=O).

3.1.3.17. 9-Ethyl-9-propyl-6,8-dioxo-3-(2-chlorophenyl)-2,3,4,5,6,7,8,9-octahydropyrimido [3,4-a]-s-triazine ***(3q)***. Yield: 32%; m.p.: 175 °C, *Anal.* (C, H, N) $C_{17}H_{21}N_4O_2Cl$. ^1H NMR ($CDCl_3$) δ ppm: 0.8 (t, 6H, 2 CH_3); 1.1 (m, 2H,$CH_3CH_2CH_2$–); 1.9 (t, 4H, CH_3CH_2– and $CH_3CH_2CH_2$–); 4.95 (s, 2H, N–CH_2–N); 5.35 (s, 2H, C=O–N–CH_2–N); 7.0 (m, 1H, Ar); 7.2 (m, 2H, Ar); 7.4 (d, 1H, Ar); 9.25 (s, 1H, ech D_2O, C=O–NH–C=O).

3.1.3.18. 9-Ethyl-9-propyl-6,8-dioxo-3-(3-chlorophenyl)-2,3,4,5,6,7,8,9-octahydropyrimido [3,4-a]-s-triazine ***(3r)***. Yield: 56%; m.p.: 144 °C, *Anal.* (C, H, N)

$C_{17}H_{21}N_4O_2Cl$. ^1H NMR ($CDCl_3$) δ ppm: 0.8 (m, 6H, 2 CH_3); 1.0 (m, 2H,$CH_3CH_2CH_2$–); 1.9 (m, 4H, CH_3CH_2– and $CH_3CH_2CH_2$–); 5.0 (s, 2H, N–CH_2–N); 5.3 (s, 2H, C=O–N–CH_2–N); 6.9 (m, 3H, Ar); 7.15 (m, 1H, Ar); 9.15 (s, 1H, ech D_2O, C=O–NH–C=O).

3.1.3.19. 9,9-Dipropyl-6,8-dioxo-3-(3-trifluoromethyl-phenyl)-7-[(3-trifluoromethyphenyl) aminomethyl]-2,3,4,5,6,7,8,9-octahydropyrimido[3,4-a]-s-triazine ***(4c)***. Yield: 46%; m.p.: 176 °C, *Anal.* (C, H, N) $C_{27}H_{29}N_5O_2F_6$. ^1H NMR ($CDCl_3$) δ ppm: 0.6 (t, 6H, 2 CH_3 propyl); 0.75 (m, 4H, $CH_3CH_2CH_2$–); 1.75 (m, 4H, $CH_3CH_2CH_2$–); 5.0 (m, 3H, N–CH_2–N and 1H, ech D_2O, NH); 5.35 (s, 4H, N–CH_2–NH and C=O–N–CH_2–N); 6.95–7.35 (m, 8H, Ar).

3.1.3.20. 9,9-Dipropyl-6,8-dioxo-3-(2-chlorophenyl)-7-[(2 - chlorophenyl)aminomethyl] - 2,3,4,5,6,7,8,9 - octa-hydropyrimido[3,4-a]-s-triazine ***(4a)***. Yield: 47%; m.p.: 127 °C, *Anal.* (C, H, N) $C_{25}H_{29}N_5O_2Cl_2$. ^1H NMR ($CDCl_3$) δ ppm: 0.7 (t, 6H, two CH_3 propyl); 0.85–1.05 (m, 4H, $CH_3CH_2CH_2$–); 1.9 (m, 4H, $CH_3CH_2CH_2$–); 4.9 (s, 2H, N–CH_2–N); 5.25 (s, 2H, C=O–N–CH_2–N); 5.4 (m, 3H, N–CH_2–NH and 1H, ech D_2O, NH); 6.7–6.9 (m, 2H, Ar); 7.05 (m, 4H, Ar); 7.2–7.4 (m, 2H, Ar).

3.1.3.21. 9,9-Dipropyl-6,8-dioxo-3-(4-trifluoromethyl-phenyl)-7-[(4-trifluoromethyphenyl) aminomethyl]-2,3,4,5,6,7,8,9-octahydropyrimido[3,4-a]-s-triazine ***(4d)***. Yield: 10%; m.p.: 182 °C, *Anal.* (C, H, N) $C_{27}H_{29}N_5O_2F_6$. ^1H NMR ($CDCl_3$) δ ppm: 0.6 (t, 6H, two CH_3 propyl); 0.75 (m, 4H, $CH_3CH_2CH_2$–); 1.75 (m, 4H, $CH_3CH_2CH_2$–); 5.0 (s, 2H, N–CH_2–N); 5.05 (m, 1H, ech D_2O, NH); 5.35 (s, 4H, N–CH_2–NH and C=O–N–CH_2–N); 6.8–7.0 (dd, 4H, Ar); 7.4–7.5 (dd, 4H, Ar).

3.1.3.22. 9 - Butyl - 9 - ethyl - 6,8 - dioxo - 3 - (2,4 - dichloro-phenyl)-7-[(2,4-dichlorophenyl) aminomethyl]-2,3,4,5, 6,7,8,9-octahydropyrimido[3,4-a]-s-triazine ***(4e)***. Yield: 13%; m.p.: 122 °C, *Anal.* (C, H, N) $C_{25}H_{27}N_5O_2Cl_4$. ^1H NMR ($CDCl_3$) δ ppm: 0.75 (dt, 6H, CH_3); 0.85 (m, 2H, $CH_3CH_2CH_2CH_2$); 1.1 (m, 2H, $CH_3CH_2CH_2CH_2$–); 1.9 (m, 4H, $CH_3CH_2CH_2CH_2$– and CH_3CH_2–); 4.85 (s, 2H, N–CH_2–N); 5.25 (s, 2H, C=O–N–CH_2–N); 5.4 (m, 3H, N–CH_2–NH and 1H, ech D_2O, NH); 6.8–7.4 (m, 6H, Ar).

3.1.3.23. 9 - Ethyl - 9 - propyl - 6,8 - dioxo - 3 - (2 - chloro-phenyl)-7-[(2-chlorophenyl) aminomethyl]-2,3,4,5,6,7, 8,9-octahydropyrimido[3,4-a]-s-triazine ***(4b)***. Yield: 15%; m.p.: 104 °C, *Anal.* (C, H, N) $C_{24}H_{27}N_5O_2Cl_2$. ^1H NMR ($CDCl_3$) δ ppm: 0.6 (t, 3H, CH_3 propyl); 0.7 (t, 3H, CH_3 ethyl); 0.9 (m, 2H, $CH_3CH_2CH_2$–); 1.85 (m, 4H, $CH_3CH_2CH_2$– and CH_3CH_2–); 4.9 (s, 2H, N–CH_2–N); 5.25 (s, 2H, C=O–N–CH_2–N); 5.4 (m, 3H, N–CH_2–NH, and 1H, ech D_2O, NH); 6.65–7.35 (m, 8H, Ar).

3.1.3.24. 9 - Ethyl - 9 - propyl - 6,8 - dioxo - 3 - (4 - chloro-phenyl)-7-[(4-chlorophenyl) aminomethyl]-2,3,4,5,6,7, 8,9-octahydropyrimido[3,4-a]-s-triazine ***(4f)***. Yield: 1%; *Anal.* (C, H, N) $C_{24}H_{27}N_5O_2Cl_2$. ^1H NMR ($CDCl_3$) δ ppm: 0.4 (t, 3H, CH_3 propyl); 0.6 (m, 5H, CH_3 propyl and $CH_3CH_2CH_2$); 1.7 (m, 4H, $CH_3CH_2CH_2$– and $CH_3CH_2CH_2$–); 4.9 (s, 2H, N–CH_2–N); 5.25 (s, 2H, C=O–N–CH_2–N); 5.6 (m, 3H, N–CH_2–NH, and 1H, ech D_2O, NH); 6.85 (m, 4H, Ar); 7.2 (m, 4H, Ar).

3.2. Biology

All compounds (except **4c**, **4e** and **4f**) have been submitted to an automated pharmatical screening.

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